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AN ANALYSIS OF THE RELEASE OF ACETYLCHOLINE FROM PREGANGLIONIC NERVE TERMINALS

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SUMMARY

1. A study has been made of the release of acetylcholine (ACh) from the preganglionic nerve terminals of the isolated superior cervical ganglion of the guinea-pig during short trains of impulses, using the amplitude of the excitatory post-synaptic potential (e.p.s.p.) as a measure of the ACh released by each impulse.

2. The time course of decay of facilitation following a single impulse could be described by two exponential components, with $\tau_1 = 200$ msec, and $\tau_2 = 13{\cdot}3$ sec. The increase in ACh output at the beginning of stimulation at frequencies $\leqslant$ 2Hz was reasonably predicted in terms of summation of the individual facilitatory effects of each impulse in the train, but fell short of the prediction at higher frequencies.

3. The steady-state output of ACh during repetitive stimulation at frequencies between 0·5 and 20 Hz was lower than that predicted by summation of the facilitatory effects of each impulse, but reached the predicted level at frequencies $\leqslant$ 2Hz in raised Mg^{2+} concentrations.

4. Statistical analysis of the quantal content (m) of e.p.s.p.s evoked by each of the first five impulses in a train showed that Poisson statistics described release of ACh at the beginning of a train in most cases; when binomial statistics could be applied (two of seven axons studied), the increase in m was accompanied by an increase in the statistical parameter, n.

5. Analyses were also made of release during continuous stimulation; at the time when the steady state of release was reached, the statistical parameter, p, had also increased. Increased release of ACh at increased frequencies of stimulation was associated with increases in p in axons with $p < 0{\cdot}5$; however, in most axons (eleven of seventeen), p was $> 0{\cdot}5$ in the steady state, and increases in m with frequency were due to increases in n.

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INTRODUCTION

At some synapses, release of transmitter may be facilitated for a few milliseconds by a preceding impulse (del Castillo & Katz, 1954*b*; Curtis & Eccles, 1960; Porter, 1970; Kuno & Weakly, 1972*a*, *b*), but during a short train of impulses a decline in the amplitude of the evoked potential is often observed (Lundberg & Quilisch, 1953*a*; del Castillo & Katz, 1954*b*; Curtis & Eccles, 1960). If the quantity of transmitter released by each impulse is reduced by raising the Mg^{2+} or lowering the Ca^{2+} concentrations, then depression of release is not seen, and successive evoked potentials increase in amplitude until a steady state of release is reached (Lundberg & Quilisch, 1953*b*; del Castillo & Katz, 1954*b*). Facilitation of release is observed at other synapses without these modifications of the normal ionic environment (Dudel & Kuffler, 1961; Burnstock & Holman, 1961; Blackman & Purves, 1969), and it has been proposed that the phenomena occur concurrently (Lundberg & Quilisch, 1953*b*; Curtis & Eccles, 1960). The release of transmitter during continuous stimulation may also be augmented by 'long-term' or 'frequency' facilitation (Maeno & Edwards, 1969), a phenomenon with properties similar to those of post-tetanic potentiation (PTP) (Magleby, 1973*a*, *b*).

In the present paper, the term 'facilitation' has been used to include both the short- and long-term increases in transmitter release which occur during nerve stimulation. A description is given of the facilitated release of acetylcholine (ACh) from mammalian preganglionic nerve terminals, using the amplitude of the excitatory post-synaptic potential (e.p.s.p.) as a measure of the ACh released per impulse. A quantitative analysis has been made using e.p.s.p.s evoked during short trains of impulses at different frequencies in normal and raised Mg^{2+} solutions. Although facilitation is marked and prolonged following a single impulse in preganglionic nerve terminals, it appears that some depression occurs during repetitive stimulation. Both facilitation and depression are increased with frequency, so that the mean ACh output per impulse is approximately constant at frequencies of 5–20 Hz during prolonged stimulation (Birks & MacIntosh, 1961; Bennett & McLachlan, 1972*b*).

Release from some mammalian preganglionic nerve terminals has been described using Poisson's Law (Sacchi & Perri, 1971); however, in many cases, failures of release are fewer than predicted, and it has been suggested that the probability of release may be high (Blackman & Purves, 1969). The model for the quantal release of transmitter proposed by del Castillo & Katz (1954*a*), in which the mean number of quanta released by an impulse, m, is given by the product of n, the number of releasable quanta, and p, their average probability of release, suggests a binomial process.

Recently, binomial statistics have been shown to describe release at several synapses (Johnson & Wernig, 1971; Wernig, 1973; Bennett & Florin, 1974), although it is not known what the statistical parameters, n and p, represent in physical terms. In the present experiments, the parameters, m (mean quantal content), n and p, have been derived from a statistical analysis of e.p.s.p.s evoked from preganglionic nerve terminals. Estimates have been made at the beginning of stimulation during early facilitation and during the steady state of transmitter release at different frequencies of stimulation. It has been shown that an increase in n occurs at the beginning of a train of impulses, while p also increases if stimulation is continued. Although p is high ($> 0{\cdot}5$) at most synapses during repetitive stimulation, and in these cases the increased ACh output at higher frequencies is due to increases in n, a significant increase in p with increases in stimulation frequency is seen at those synapses where p is initially low (see also Wernig, 1972*a*).

METHODS

Isolated superior cervical ganglia from guinea-pigs (150–250 g) were used in all experiments. The experimental arrangement and techniques were the same as those described previously (Bennett & McLachlan, 1972*a*). Excitatory post-synaptic potentials (e.p.s.p.s) were recorded in ganglion cells in response to stimulation of the cervical sympathetic trunk, the stimulus strength being adjusted so that only one axon was being excited. Responses were analysed only if the largest e.p.s.p. amplitudes during repetitive stimulation did not exceed about 10 mV, the majority of e.p.s.p.s being much smaller. The observed resting potentials (between 50 and 70 mV) measured using high-resistance micro-electrodes may be unreliable; under these conditions, the correction for non-linear summation of potentials (Martin, 1955) was not applied.

A description of the decay of facilitation after a single impulse was derived from the responses to pairs of conditioning-test stimuli. Facilitation was defined as $(v-v_0)/v_0$, where v is the amplitude of the test e.p.s.p., and v_0 the amplitude of the conditioning e.p.s.p. (Mallart & Martin, 1967). The amplitude of the response to the conditioning impulse in a single terminal was very variable, so it was necessary to pool the results from more than 10 for each conditioning-test interval. At least $1\frac{1}{2}$ min of rest was allowed between pairs of stimuli, at least 5 min after trains of one hundred impulses, and longer after prolonged stimulation, in order that the effects of previous activity could wear off.

Min. e.p.s.p.s recorded in a cell during and for a few seconds after repetitive stimulation were assumed to originate from the stimulated axon (Blackman & Purves, 1969), and were used to evaluate the mean and variance of the quantal size. Very large or obviously multiquantal responses were excluded; these represented fewer than 1 % of min. e.p.s.p.s observed at synapses examined in this study (cf. Bornstein, 1974). The resting frequency of spontaneous potentials in autonomic ganglion cells is low (usually less than 3/min) (Blackman & Purves, 1969; Dennis, Harris & Kuffler, 1971), so that the inclusion of spontaneous synaptic potentials originating from unstimulated axons is not likely to distort the population significantly. It was assumed that the unit size is not altered during stimulation (del Castillo & Katz, 1954*b*; Martin & Pilar, 1964*a*).

Normally at least fifty consecutive evoked responses were selected for application of binomial statistics in conditions of steady-state release (determined by comparison of the first and last ten responses using Student's t test). In a few cases, the analysis was carried out on smaller populations when only limited data were available; the calculated standard errors of the estimates incorporated this source of error.

Amplitude-frequency histograms of e.p.s.p.s were drawn and compared with predictions derived from statistical principles as outlined below. The bin size for evoked e.p.s.p. histograms was selected as the size of the quantal unit for that synapse, in order to simplify the calculations on relatively small populations of data. The χ^2 test for goodness of fit was applied to determine which prediction gave better agreement with the observed data, using three degrees of freedom less than the number of bins for binomial predictions and two degrees of freedom less than the number of bins for the other distributions.

Binomial analysis. The hypothesis that $m = np$ (del Castillo & Katz, 1954*a*) implies that the probability that r quanta are released by each impulse, $P(r)$, will be given by the binomial expression

$$P(r) = \binom{n}{r} p^r q^{n-r},$$

where $q = (1-p)$. When individual quanta can be measured, the binomial theory gives

$$p = 1-\frac{s^2}{m} \quad \text{and} \quad n = \frac{m}{p}$$

(Ginsborg, 1970), where m is the mean number of quanta released by each impulse, and s^2 is the variance of the distribution.

If the quantal size is not uniform, but direct estimates can be made of the mean, $\bar{x}$, and variance, σ^2, of the spontaneous potentials, then this variation must be included in the estimates, and

$$p = 1-\frac{S^2}{m\bar{x}^2}+\frac{\sigma^2}{\bar{x}^2},$$

where S^2 is the variance of the evoked potentials, and m is the mean quantal content (= mean evoked potential amplitude/$\bar{x}$). Furthermore, the probability distribution of evoked potentials, $P(r)$, is not simply the binomial expansion, but must be modified to include the distribution of quantal size. At the neuromuscular junction, where the distribution of quantal units can be described by a normal distribution (del Castillo & Katz, 1954*a*; Boyd & Martin, 1956), the binomial probability distribution of evoked potentials is given by

$$P(X) = \sum_{r=0}^{n} \binom{n}{r} p^r q^{n-r} \, (2\pi r\sigma^2)^{-\frac{1}{2}} \, e^{(X-\bar{x}r)^2/2r\sigma^2}$$

(Bennett & Florin, 1974). In the Results, evidence is given to support the idea that the quantal unit released from preganglionic terminals is described not by a normal distribution, but rather by a gamma distribution. This distribution differs from a normal distribution by being somewhat skewed (e.g. Fig. 5*A*, *a*), and has the frequency function,

$$f(x) = \frac{\lambda^k}{\Gamma(k)} e^{-\lambda x} x^{k-1},$$

where $\lambda = \bar{x}/\sigma^2$ and $k = \lambda\bar{x}$. $\Gamma(k)$ is the gamma function, which is equal to $(k-1)!$ for integer values of k, and can be evaluated from tables (Pearson & Hartley, 1958) for non-integer values. This distribution can be summed in the same way as the normal

distribution, so that the binomial probability distribution of evoked potentials with a gamma-distributed unit is

$$P(X) = \sum_{r=0}^{n} \binom{n}{r} p^r q^{n-r} \frac{\lambda^{kr}}{\Gamma(kr)} e^{-\lambda X} X^{(kr-1)}.$$

The mean quantal content, m, is again determined from the mean evoked potential amplitude/$\bar{x}$.

Poisson's Law gives the probability distribution of the release of r quanta,

$$P(r) = \sum_{r=0}^{n} \frac{e^{-m} n^r}{r!},$$

which can also be modified to account for the quantal size variation (see Blackman, Ginsborg & Ray, 1963*b*).

The standard error of the estimate of m is given by

$$\text{S.E.}^2_m = \frac{S^2}{\bar{x}^2 N} + \frac{m^2 \bar{x}^2}{\bar{x}^2 M},$$

for N evoked potentials and M spontaneous potentials (cf. Martin, 1966). The standard errors of the estimates of n and p for gamma-distributed units have been calculated from equations derived by Dr J. Robinson.

RESULTS

Release of ACh at the beginning of a train of impulses

E.p.s.p.s recorded in ganglion cells during repetitive stimulation of a single preganglionic axon increased in amplitude at the beginning of a train at all frequencies (0·1–20 Hz). If stimulation was continued, especially at high frequencies, a later decline in e.p.s.p. amplitude occurred over minutes; this has been attributed to depletion of the main store of ACh (Bennett & McLachlan, 1972*a*, *b*).

A semilogarithmic plot of facilitation at different conditioning-test intervals (Fig. 1) describes the time course of its decay after a single impulse, this curve was analysed into two exponential components, one having a value of facilitation (f_1) of 0·4 at zero time, and lasting for about 300 ms; a slower component of similar size (f_2 = 0·45) decayed over more than ten seconds. If it is assumed that the facilitatory effects produced by each impulse simply add to each other (Mallart & Martin, 1967), then the growth of facilitation during a train should be described by $f = f_1 e^{-b_1 t} + f_2 e^{-b_2 t}$, where b_1 is the rate constant of decay of the first component (5·0), and b_2 is the rate constant of decay of the second component (0·075). The exponential growth of facilitation during trains of impulses at different frequencies was predicted using this equation. At frequencies $\leqslant$ 2 Hz, the mean experimental values provided a good fit to the predicted values (Fig. 2*a*), at least for the first few impulses. With frequencies of stimulation greater than 2 Hz, the mean experimental values diverged progressively more from those predicted after only a few impulses (Fig. 2*b*). It appears

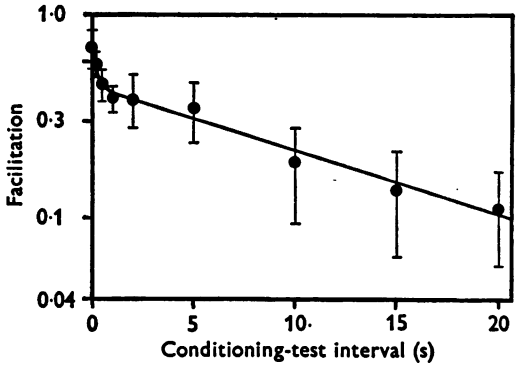

Fig. 1. The time course of decay of facilitation. Values of f were obtained from the responses to pairs of conditioning-test stimuli, facilitation (f) being defined as $(v-v_0)/v_0$, where v is the amplitude of the test e.p.s.p. and v_0 the amplitude of the conditioning e.p.s.p. The filled circles represent the mean values at each time interval measured in different cells ($n \geqslant 10$); the bars indicate the S.E. of the means of these points. The facilitation produced by a conditioning impulse decays over more than half a minute, with two exponential components, one fast (τ_1 = 200 msec), and the other lasting many seconds (τ_2 = 13·3 sec).

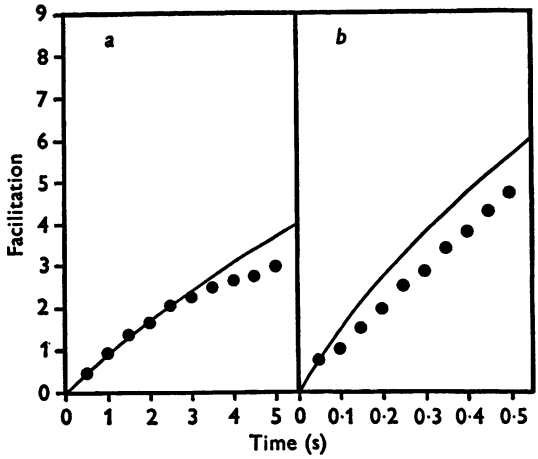

Fig. 2. A comparison of the growth of facilitation observed at the beginning of stimulation with that predicted on the basis of summation of the facilitatory effects of each impulse. Frequency of stimulation in (*a*) 2 Hz, (*b*) 20 Hz. The curves represent the predicted growth of facilitation calculated from equations described in the text. The filled circles represent the mean experimental values obtained in normal perfusion medium, the S.E. of the means being less than $\pm 19\%$ (n = 19 in (*a*), 18 in (*b*)). The observed values follow those predicted for the first few impulses and then fall below them progressively more, the discrepancy being greater at the higher frequency.

that, at the beginning of a short train of impulses in preganglionic nerve terminals, the facilitatory effects of each impulse add to each other, but at high frequencies of stimulation there is some concomitant depression of ACh release.

The release of ACh during repetitive stimulation at different frequencies

During continuous stimulation of the preganglionic axon, a steady state of ACh release was reached which was maintained for several hundred impulses at low frequencies ($\leqslant$ 2 Hz). At higher frequencies there was a gradual decline in output after an initial peak until a plateau level was reached at between 15 and 30 sec of stimulation (Fig. 3). The steady-state

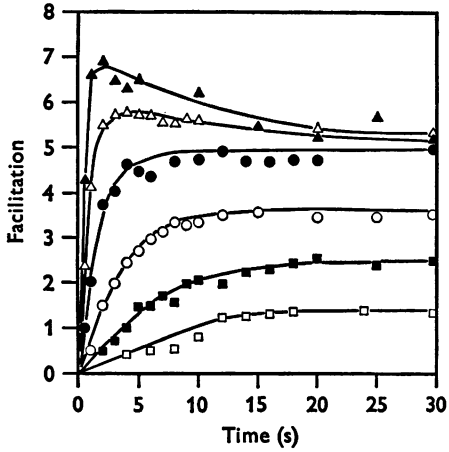

Fig. 3. The changes in facilitation during 30 s of continuous stimulation. The symbols represent mean values obtained at different frequencies at intervals after the beginning of stimulation, the standard errors of the means always being less than $\pm 18\%$ ($n \geqslant 11$). Frequencies of stimulation: filled triangles, 20 Hz; open triangles, 10 Hz; filled circles, 5 Hz; open circles, 2 Hz; filled squares, 1 Hz; open squares, 0·5 Hz. At the lower frequencies, facilitation increases and reaches a plateau level which is proportional to the frequency. At the higher frequencies, depression is superimposed on the facilitation, to increasing degrees with increasing frequency, so that at frequencies $\geqslant$ 5 Hz the facilitated ACh release is the same after about 15 sec of stimulation.

release predicted from the summation of the facilitatory effects of each impulse ($f_1(e^{b_1 t}-1)^{-1}+f_2(e^{b_2 t}-1)^{-1}$, Mallart & Martin, 1967) was never reached in normal solutions, although the discrepancy was less at the lower frequencies (Fig. 4). As a result of the gradual decline in the amount released at high frequencies, the ACh output per impulse at about 15–30 sec after the beginning of stimulation became approximately constant at frequencies $\geqslant$ 5 Hz (Fig. 3) (see also Birks & MacIntosh, 1961; Bennett

& McLachlan, 1972*b*), while it was proportional to frequency at the lower rates. The failure of the terminal to achieve the predicted levels of output at high frequencies may be attributed either to the inability of the release mechanism to maintain high release rates, or to a depletion of available transmitter.

The effect of high Mg^{2+} concentrations on ACh release

If the amount of ACh released by each impulse is decreased by the addition of Mg^{2+} ions to the perfusion medium (del Castillo & Katz, 1954*a*), then it might be expected that depletion of transmitter would be reduced. The release of ACh from preganglionic nerve terminals was therefore examined in the presence of 5–15 mM-$MgCl_2$.

Increasing the Mg^{2+} concentration from 1·2 to 15 mM led to an increase in the input resistance of most ganglion cells (input resistance 53·95 ± 5·32 MΩ, S.E. of mean, n = 21, cf. control input resistance 34·13 ± 1·37 MΩ, S.E. of mean, n = 115) and the min. e.p.s.p.s were of larger amplitude; it was thus difficult to detect much decrease in the amplitude of the e.p.s.p.s. An increased threshold for firing of action potentials was also observed.

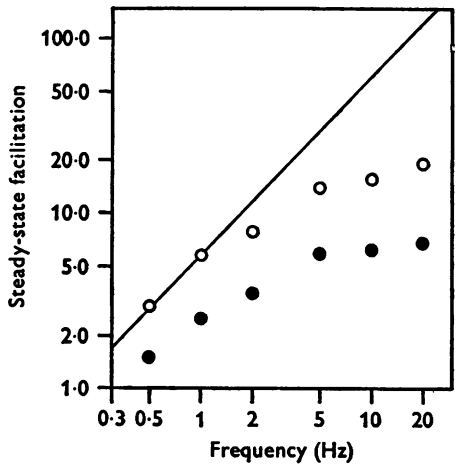

Fig. 4. A comparison between the steady-state levels of facilitation observed in normal and raised Mg^{2+} solutions and that predicted on the basis of summation of the facilitatory effects of each impulse. The filled circles are the mean values obtained in different cells in normal perfusion medium, the S.E. being less than ± 17 % ($n \geqslant 12$). The open circles are the mean values obtained in solutions containing an additional 15 mM-$MgCl_2$, the standard errors ranging from less than ± 13 % at low frequencies to ± 28 % at high frequencies ($n \geqslant 4$). The steady-state values are consistently below the predicted line at high frequencies; raising the Mg^{2+} concentration increased all values and at the low frequencies provided a reasonable agreement with the predicted values.

The amount of facilitation produced by a conditioning impulse of the response to a test impulse was not significantly different from that observed in normal solutions, as has been reported for adrenergic terminals (Bennett, 1973). Comparison of the steady-state ACh output in the presence of additional Mg^{2+} ions with the predictions based on the summation of the facilitatory effects of each impulse showed good correspondence at frequencies $\leqslant$ 2 Hz (Fig. 4), but still fell short at higher frequencies. It should be noted that the measurement of facilitated outputs at high frequencies in these solutions was inaccurate, as e.p.s.p. amplitudes varied widely and often exceeded 10 mV under these conditions. The effect of raised Mg^{2+} concentrations of increasing ACh release toward the values predicted for simple summation of facilitation suggests that some depletion of the store of transmitter occurs during short trains of impulses, and this may be responsible for the relative depression of ACh release in normal solutions.

Binomial analysis of ACh release

The quantal unit released from preganglionic nerve terminals. It has been shown (Sacchi & Perri, 1971) that the min. e.p.s.p.s recorded in mammalian ganglion cells correspond to the unit evoked potentials when m is low; the population of min. e.p.s.p.s can therefore be taken as a measure of quantal size. The amplitude-frequency distribution of min. e.p.s.p.s recorded during stimulation of a single preganglionic axon is positively skewed (see Figs. 5, 6, 7). The shape of this distribution is more like that of a gamma distribution than a normal distribution (see Methods). Statistical methods have been applied to determine whether the gamma distribution provides a better description of the quantal unit variation at preganglionic nerve terminals.

In twenty-two ganglion cells, min. e.p.s.p. amplitude distributions were fitted better by gamma distributions than normal distributions, using the χ^2-test for goodness of fit ($P > 0{\cdot}1$). In three cells, normal distributions were as good in describing the spontaneous potential populations (Table 1). In ten other cells, neither distribution provided an adequate fit with the observed data, mainly because of the small numbers of min. e.p.s.p.s recorded, and all data from these cells were rejected for further analysis. Min. e.p.s.p. amplitude distributions recorded in autonomic ganglion cells by other authors have also been analysed; gamma distributions provided better fits with these published observations than did normal distributions (see Table 2). The variation of the quantal unit released from a preganglionic axon in terms of a gamma frequency function was therefore incorporated in the equations to be applied in the examination of evoked transmitter release (see Methods).

TABLE 1. Values of the parameters, λ and k, for gamma distributions derived from populations of min. e.p.s.p.s, together with results of the χ^2-test for goodness of fit to such distributions compared with fit to normal distributions. P, probability that the population could be a sample from the predicted distribution. M, number of observations

Axon	M	λ	k	P (gamma)	P (normal)
I	71	29·95	19·45	> 0·5	> 0·2
II	176	21·91	12·29	> 0·1	< 0·01
III	114	18·57	11·98	> 0·9	> 0·5
IV	331	18·88	9·97	> 0·5	< 0·01
V	122	12·06	5·55	> 0·1	< 0·01
VI	79	21·89	12·94	> 0·5	> 0·1
VII	137	9·15	7·92	> 0·5	> 0·2
VIII	92	19·24	11·22	> 0·5	> 0·1
IX	83	18·27	15·39	> 0·5	> 0·05
X	54	18·36	12·04	> 0·7	> 0·2
XI	53	17·75	10·15	> 0·1	> 0·02
XII	172	11·68	7·61	> 0·1	> 0·01
XIII	128	30·30	19·91	> 0·2	> 0·02
XIV	159	8·97	8·28	> 0·5	< 0·01
XV	33	16·12	16·89	> 0·5	> 0·1
XVI	96	12·66	13·14	> 0·1	< 0·01
XVII	111	12·04	11·65	> 0·05	> 0·01
XVIII	29	42·43	31·61	> 0·98	> 0·9
XIX	24	16·79	14·20	> 0·8	> 0·5
XX	31	21·33	11·07	> 0·99	> 0·9
XXI	41	17·23	13·16	> 0·9	> 0·8
XXII	28	20·88	12·82	> 0·2	> 0·3
XXIII	27	7·27	6·81	> 0·1	> 0·05
XXIV	120	21·37	15·37	> 0·5	> 0·05
XXV	84	38·10	22·94	> 0·5	> 0·3

Evoked release at the beginning of a train of impulses

In order to determine the values of m, n and p during the facilitation of release at the beginning of a train of impulses, short trains of five stimuli at 2 Hz were applied to a preganglionic axon. This frequency was used because the facilitated ACh release during the first few impulses is well described by the summation of the facilitation produced by each successive impulse (Fig. 2), and depression of release is not observed.

As many short trains as possible were recorded, together with a longer train to determine the release parameters in the steady state; as intervals of 3 min had to be allowed between trains, sufficient numbers for analysis were obtained from only seven axons, all of which showed an increase in m over the first five impulses. In five of these axons, the values of p during the first five impulses determined using the binomial theory were very low and the errors so high as to make the estimates meaningless; in these

cases, the amplitude–frequency histograms of e.p.s.p.s for each impulse were well fitted by Poisson distributions (Fig. 5*A*). However, during the steady state of release at 2 Hz values of p of about 0·5 were obtained and the evoked potential distributions could be fitted using the binomial equations (Fig. 5*A*). In the other two axons, it was possible to apply binomial statistics to analyse release at the beginning of stimulation. In both these cases, although no significant change in p could be detected, an increase in n occurred over the first five impulses. A further increase in m at the time when the steady state was reached was accompanied by increases in both n and p (Fig. 5*B*). In these experiments, while an increase in n seemed to be the main factor contributing to early facilitation, at least at synapses where release by a single impulse had a high value of p (cf. Zucker, 1973), an increase in p was observed if stimulation was continued.

TABLE 2. Results of the χ^2-test for goodness of fit to gamma and normal distributions of published amplitude–frequency histograms of min. e.p.s.p.s recorded in autonomic ganglion cells. P, probability that the population could be a sample from the predicted distribution

Authors	Preparation	Figure	P (gamma)	P (normal)
Blackman, Ginsborg & Ray (1963*a*)	Frog sympathetic ganglion	5*b*	> 0·3	> 0·3
		5*c*	> 0·9	> 0·5
		5*e*	> 0·7	< 0·01
Martin & Pilar (1964*a*)	Chick ciliary ganglion	7*A*	> 0·5	> 0·5
		7*B*	> 0·1	< 0·01
		7*C*	> 0·1	< 0·01
Blackman & Purves (1969)	Guinea-pig paravertebral ganglion	13	> 0·2	> 0·02
Dennis, Harris & Kuffler (1971)	Frog parasympathetic ganglion	16*b*	> 0·2	> 0·01
Sacchi & Perri (1971)	Guinea-pig superior cervical ganglion	3	> 0·9	> 0·7
		5	> 0·8	> 0·7

In Figs. 5*d*, 5*f* (Blackman, Ginsborg & Ray, 1963*a*) and Fig. 7*D* (Martin & Pilar, 1964*a*), an iterative procedure using the gamma distribution provides evidence for multiquantal release (J. Bornstein, personal communication).

Evoked release in the steady state at different frequencies

A statistical analysis was also made of release during the steady state at different frequencies of stimulation. The values of m, n and p, determined from experiments on seventeen presynaptic axons, each stimulated at more than one frequency, are shown in Table 3. In three cases the value of p was low, or below the limits of the estimation, during stimulation at low frequencies (axons I–III); however, at higher frequencies p had values > 0·5 and the estimates of n and p became meaningful. In three other

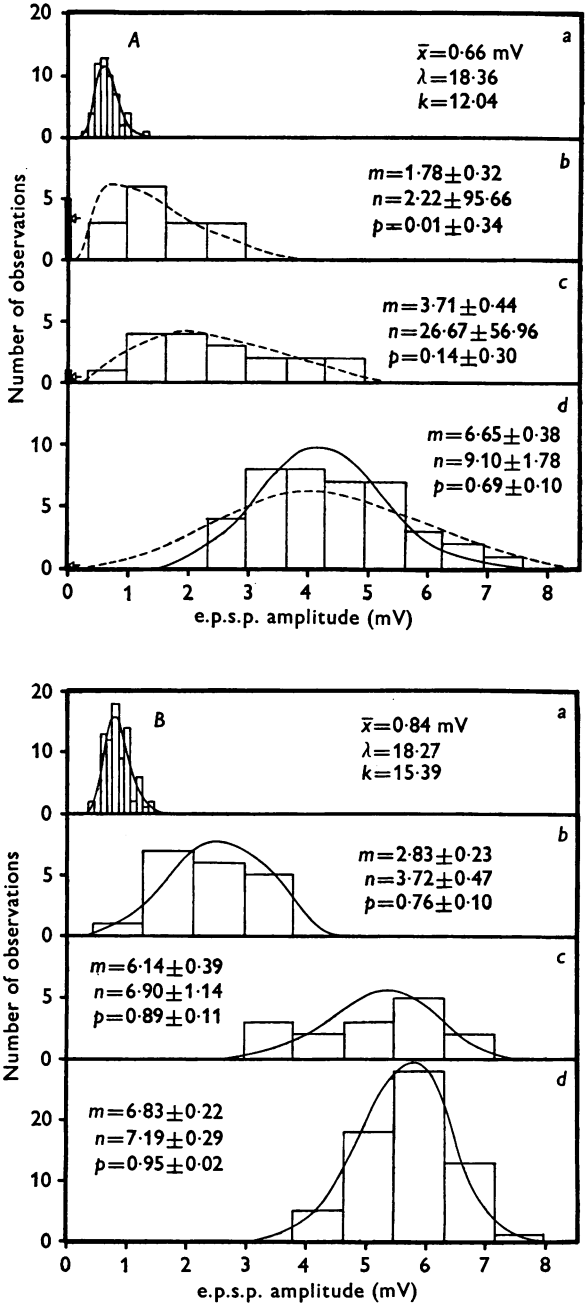

Fig. 5. For legend see facing page.

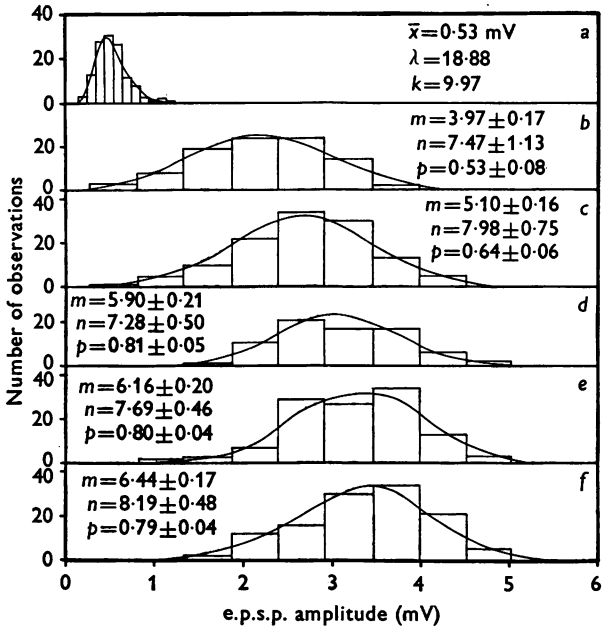

Fig. 6. The effect of frequency of stimulation on release parameters in an axon showing changes in p (axon IV). Amplitude–frequency histograms of e.p.s.p.s recorded during stimulation at different frequencies. *a*, min. e.p.s.p.s; curve represents Γ-distribution calculated from these data. *b–f*, e.p.s.p.s evoked during steady-state release at 1 Hz (*b*), 2 Hz (*c*), 5 Hz (*d*), 10 Hz (*e*), 20 Hz (*f*). Continuous lines (*b–f*) represent predicted release based on binomial statistics. The value of m increases with increases in frequency, although little difference is observed at frequencies $\geqslant$ 5 Hz; this change is due to changes in p, n remaining relatively constant.

Fig. 5. The changes in release parameters at the beginning of a train of impulses. Amplitude–frequency histograms of e.p.s.p.s recorded during stimulation of two preganglionic axons (*A*, *B*) at 2 Hz. *a*, min. e.p.s.p.s; curves represent the Γ-distributions calculated from these data which were used to predict evoked release. *b*, *c*, e.p.s.p.s evoked by the 1st and 5th impulses of trains, and *d*, during steady-state release. Interrupted lines represent predicted release based on Poisson statistics, continous lines that based on binomial statistics. In *A* (axon X), release by the first few impulses is well fitted using Poisson's Law, and the parameter, p, is low; however, during steady-state release binomial statistics provide a better description of release. In *B* (axon IX), all populations of evoked potentials are reasonably described using binomial statistics; m and n increase as release is facilitated at the beginning of repetitive stimulation, while p is also increased by the time the steady state is reached.

TABLE 3. Estimates of release parameter frequencies of stimulation

Axon	Frequency of stimulation (Hz)	$m \pm$ S.E.$_m$	$n \pm$ S.E.$_n$	$p \pm$ S.E.$_p$
I	2	3·83 ± 0·23	—	(−0·03)
	10	9·31 ± 0·36	17·77 ± 3·04	0·52 ± 0·09
II	1	7·36 ± 0·39	—	(−0·03)
	2	18·32 ± 0·60	38·80 ± 9·54	0·47 ± 0·12
III	1	3·32 ± 0·21	28·41 ± 35·0	0·12 ± 0·14
	2	3·71 ± 0·25	12·89 ± 5·94	0·29 ± 0·13
	10	5·28 ± 0·20	7·23 ± 0·58	0·73 ± 0·05
IV	1	3·97 ± 0·17	7·47 ± 1·14	0·53 ± 0·08
	2	5·10 ± 0·16	7·98 ± 0·75	0·64 ± 0·06
	5	5·90 ± 0·21	7·28 ± 0·50	0·81 ± 0·05
	10	6·16 ± 0·20	7·69 ± 0·46	0·80 ± 0·04
	20	6·44 ± 0·17	8·19 ± 0·48	0·79 ± 0·04
V	2	10·65 ± 0·49	15·48 ± 2·06	0·69 ± 0·08
	10	12·55 ± 0·54	14·76 ± 1·25	0·85 ± 0·06
VI	1	4·93 ± 0·29	13·94 ± 5·27	0·35 ± 0·13
	2	7·25 ± 0·35	17·06 ± 4·49	0·43 ± 0·11
	10	12·75 ± 0·49	20·53 ± 2·74	0·62 ± 0·08
	20	12·98 ± 0·53	16·81 ± 1·75	0·77 ± 0·08
VII	2	3·83 ± 0·15	4·08 ± 0·21	0·94 ± 0·03
	5	7·77 ± 0·27	7·99 ± 0·36	0·97 ± 0·02
	10	8·02 ± 0·28	9·26 ± 0·54	0·87 ± 0·04
	20	8·11 ± 0·29	10·16 ± 0·73	0·80 ± 0·05
VIII	2	11·12 ± 0·50	11·37 ± 0·71	0·98 ± 0·03
	10	12·93 ± 0·56	12·68 ± 0·67	1·02 ± 0·02
	20	9·64 ± 0·46	12·11 ± 1·19	0·80 ± 0·06
IX	2	6·83 ± 0·22	7·19 ± 0·29	0·95 ± 0·02
	10	6·85 ± 0·21	7·03 ± 0·25	0·98 ± 0·02
X	2	6·65 ± 0·38	10·55 ± 1·78	0·63 ± 0·10
	10	12·55 ± 0·56	19·21 ± 2·40	0·65 ± 0·07
XI	2	17·28 ± 0·80	19·04 ± 1·32	0·91 ± 0·04
	10	20·68 ± 0·94	29·58 ± 3·17	0·70 ± 0·06
XII	1	10·16 ± 0·39	13·30 ± 1·34	0·76 ± 0·07
	2	10·69 ± 0·41	13·55 ± 1·30	0·79 ± 0·07
	5	14·83 ± 0·50	18·72 ± 1·56	0·79 ± 0·06
	10	12·31 ± 0·39	14·29 ± 0·83	0·86 ± 0·04
XIII	1	6·78 ± 0·22	12·27 ± 1·53	0·55 ± 0·07
	2	9·47 ± 0·28	18·25 ± 2·49	0·52 ± 0·07
	5	11·52 ± 0·38	23·33 ± 4·48	0·49 ± 0·09
	10	12·09 ± 0·40	24·15 ± 4·62	0·50 ± 0·10
XIV	1	3·11 ± 0·15	3·64 ± 0·26	0·86 ± 0·05
	2	5·28 ± 0·20	6·53 ± 0·46	0·81 ± 0·05
	5	5·25 ± 0·19	6·85 ± 0·46	0·77 ± 0·04

TABLE 3 (*cont.*)

Axons	Frequency of stimulation (Hz)	$m \pm$ S.E.$_m$	$n \pm$ S.E.$_n$	$p \pm$ S.E.$_p$
XV	2	3·85 ± 0·21	5·03 ± 0·43	0·77 ± 0·05
	10	6·63 ± 0·32	8·07 ± 0·59	0·82 ± 0·04
XVI	5	4·97 ± 0·16	4·96 ± 0·18	1·00 ± 0·01
	10	5·73 ± 0·18	5·85 ± 0·22	0·98 ± 0·02
	20	6·90 ± 0·22	7·16 ± 0·28	0·96 ± 0·02
XVII	1	3·17 ± 0·14	3·54 ± 0·20	0·90 ± 0·04
	2	3·83 ± 0·15	4·00 ± 0·19	0·96 ± 0·03
	5	4·12 ± 0·13	4·11 ± 0·16	1·00 ± 0·01
	10	6·30 ± 0·23	7·52 ± 0·46	0·84 ± 0·04

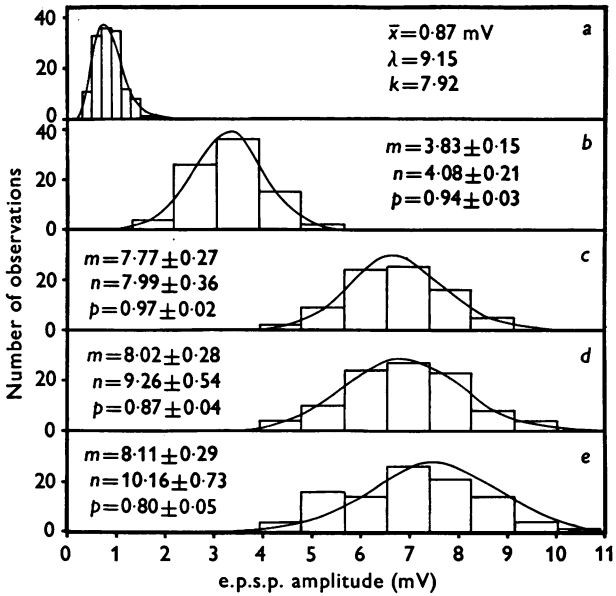

Fig. 7. The effect of frequency of stimulation on release parameters in an axon showing changes in n (axon VII). Amplitude–frequency histograms of e.p.s.p.s recorded during stimulation at different frequencies. *a*, min. e.p.s.p.s; curve represents Γ-distribution calculated from these data. *b–e*, e.p.s.p.s evoked during steady-state release at 2 Hz (*b*), 5 Hz (*c*), 10 Hz (*d*), 20 Hz (*e*). Continuous lines (*b–e*) represent predicted release based on binomial statistics. The value of m increases with frequency, and is associated with increases in n, rather than changes in p.

axons, p increased with increases in frequency (Fig. 6); little change in n occurred in two of these, while in the third, both p and n increased (axon IV). However, in the majority of axons (eleven of seventeen), p was > 0·5,

even at low frequencies, and little change in p was observed with changes in frequency. Increased ACh release during stimulation at higher frequencies was associated primarily with increases in n (Fig. 7). The data were divided into two groups, one (axons I–VI) with little change in n at different frequencies, and the other (axons VII–XVII) having relatively constant values of p; the values for m, n and p were normalized to the value at 2 Hz for each axon. The pooled results for each group were averaged and can be seen in Fig. 8. In all of axons I–VI, p was $> 0{\cdot}5$ at low frequency, and increases in m were accompanied by increases in p, while in the

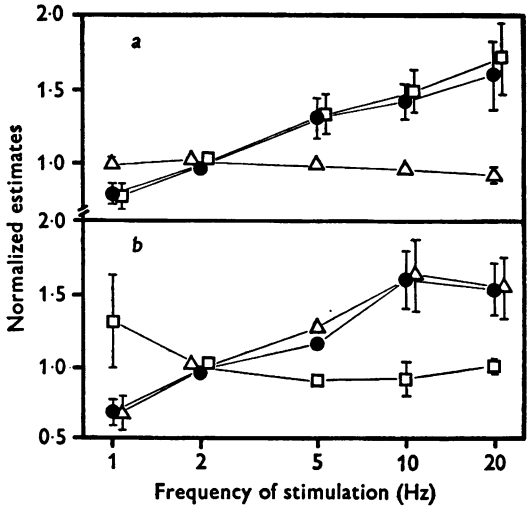

Fig. 8. The changes in release parameters in two groups of axons. Values normalized to the value at 2 Hz and pooled for each group. Filled circles represent m, open triangles represent p and open squares represent n. The vertical bars indicate the S.E. of the means. In *a* (axons VII–XVII), p is high and constant and changes in m are due to changes in n, while in *b* (axons I–IV) where $p < 0{\cdot}5$, increases in m are associated with increases in p, n remaining relatively constant.

remaining axons, all with $p > 0{\cdot}5$, changes in m of similar magnitude were always related to changes in n. These results suggest that different preganglionic axons have different values of p for transmitter release by a single impulse, and that the facilitated release of ACh during trains of impulses may be associated with increases in both n and p.

DISCUSSION

The results of this study have confirmed that, after a single impulse in a preganglionic nerve, there is facilitation of the post-synaptic response which lasts for several seconds (Larrabee & Bronk, 1947; Job & Lundberg,

1953). This facilitation is due to an increase in the number of quanta released by successive impulses, rather than to alteration of post-synaptic sensitivity, as at other synapses (del Castillo & Katz, 1954*b*; Liley, 1956; Martin & Pilar, 1964*b*). A component of facilitation after a single impulse which lasts more than one hundred milliseconds occurs at most synapses which have been studied (Martin & Pilar, 1964*b*; Thies, 1965; Mallart & Martin, 1967; Bennett, 1973; Linder, 1973), while a more prolonged component of several seconds' duration has been reported at adrenergic nerve terminals (Bennett, 1973). At Mg^{2+}-blocked amphibian neuromuscular junctions, there is a small prolonged component of facilitation due to a single impulse, which is frequency-dependent and has the same properties as PTP (Magleby, 1973*a*, *b*). Whether the second component at autonomic synapses represents a similar potentiation phenomenon, independent of the shorter component of facilitation, cannot be determined from the present data, as the development of an increased output during a train appears to be limited by the availability of transmitter from the bulk stores (see also Bennett, 1973). The output of ACh from preganglionic nerve terminals during repetitive stimulation results from a combination of the effects of both components of facilitation together with some concurrent depletion, and 'steady-state release' can thus only be defined relative to unstimulated conditions.

The quantal unit at neuromuscular junctions innervated by a single axon can be described by a normal distribution (del Castillo & Katz, 1954*a*; Boyd & Martin, 1956), but it has not been possible to examine the variation in quantal size at individual synapses between neurones other than in autonomic ganglia. The quantal unit at ganglionic synapses varies in a way which is described better by a gamma distribution than by a normal distribution. It seems unlikely that this results from distortion of potentials during propagation along dendrites (McLachlan, 1974), or from polyneuronal synaptic inputs, as the same distribution can be fitted to the populations of min. e.p.s.p.s recorded in amphibian autonomic and chick ciliary ganglia (see Table 2), both of which contain monopolar ganglion cells with single preganglionic axons. Furthermore the normal distribution is approximated by a gamma distribution with $k \rightarrow 30$, and so may only represent a special case. A similarity between skewed distributions of spontaneous potential amplitudes and synaptic vesicle volumes at mammalian neuromuscular junctions suggests that quantal size variation may be related to the population of synaptic vesicles in the nerve terminals.

Binomial statistics have been used to describe the release of ACh evoked by nerve impulses from normal mammalian preganglionic nerve terminals in the absence of modification of the ionic environment. While this investigation has been limited to axons which produced small subthreshold

e.p.s.p.s even during repetitive stimulation, failures of release from those axons which produce supra-threshold e.p.s.p.s are never seen; it seems likely then that p is high at the majority of synapses in these ganglia. In cases where p is small ($< 0{\cdot}2$), the errors of the estimates of n and p become very large, and Poisson statistics can be applied and provide a good description of release (see also Johnson & Wernig, 1971). It seems likely that p is reduced in high Mg^{2+} and low Ca^{2+} concentrations (Wernig, 1972*b*; Bennett & Florin, 1974); the demonstration of Poisson release under these conditions is therefore consistent with the theory that the normal release of transmitter is binomial (Blackman, Ginsborg & Ray, 1963*b*; Christensen & Martin, 1970).

Studies of facilitated release during the first few impulses of a train at an inhibitory synapse on the mammalian somatic motorneurone (Kuno & Weakly, 1972*b*), and at regenerating mammalian somatic neuromuscular junctions (Bennett & Florin, 1974), suggested that there was an increase in n rather than a change in p. This has also been observed at two synapses in the present study. However, during repetitive stimulation, p had also increased by the time that the steady state of release was reached. At increased frequencies of stimulation p increased further unless it was already high ($p > 0{\cdot}5$) (see Wernig, 1972*a*), when increases in m were mainly due to increases in n. Although early facilitation has been attributed to either an increase in the probability of release of transmitter with successive impulses, 'p' (del Castillo & Katz, 1954*b*; Mallart & Martin, 1968), or to a rapid transient increase in available transmitter, 'n', by mobilization from bulk stores (Curtis & Eccles, 1960; Hubbard, 1963), it is not clear whether there is any relationship between these concepts and the statistical parameters, p and n, derived using binomial statistics. Despite these uncertainties, the fact that such a statistical model can be applied implies that, in terms of quanta, the definitions of del Castillo & Katz (1954*a*) are tenable. The present data are consistent with the notion that early facilitation is primarily related to an increase in the parameter, n (Bennett & Florin, 1974), while the long-term effects of repetitive stimulation also involve an increase in the parameter, p. It is hoped that further analyses of release at synapses in mammalian ganglia can help to elucidate the role of these statistical parameters in the normal release of transmitter.

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